Effects of Methane Inhibition and BCVFA Supplementation on Production of Fatty Acids in Continuous Culture Fermenters

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Department of Animal Sciences

The Ohio State University

by Vianca Natalia Hernandez Rosado

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Project Advisors: Dr. Jeffery Firkins, Department of Animal Science

Abstract

Ruminants, such as cows, use microorganisms to digest fiber in order to absorb nutrients. As the microbes digest fiber they create H_2 which the methanogens (a type of archaea) use to make methane. Methane produced by ruminants is not only a source of greenhouse gasses in the atmosphere, about 3% of the total methane produced, but also is potentially wasted metabolizable energy. There is current research on the relationship of methane inhibition in bacteria, suggesting the movement of aqueous hydrogen (hydrogen has been proven to increase with methane inhibition) helps in the production of longer chain fatty acids, such as valerate and caproate (Hristov, 2015). Longer chain fatty acids are used in the cell of bacteria to make the cell membrane more rigid, while branched chain fatty acids are used to make the cell membrane more flexible. To test the effects of inhibiting methane production, the following four artificial rumens continuous culture fermenters with 4 different treatments; a 3-nitrooxipropanol (NOP), a branched chain violate fatty acids (BCVFA), one treated with both NOP and BCVFA and a control with no added treatment. During a 3 day period samples for VFA analysis, fiber analysis and methane analysis were collected. We expected that the NOP group would decrease methane production, increase the amount of longer chain fatty acids produced, and increase the fiber digestibility. The BCVFA group will be expected to increase fiber digestibility, while the group treated with both NOP and BCVFA would increase the rate of the combined previous outcomes. These findings would indicate that inhibiting methane would increase the formation of longer chain fatty acids; subsequently, bacteria will grow and reproduce more rapidly allowing the cow to

digest more bacteria and receive more metabolizable energy. This study could help decrease the amount of methane created by cows while improving overall production.

Introduction

Ruminants, such as cows, are host to microorganisms that break down fiber and produce energy for the cow. As part of the fermentation process, waste products such as methane (CH_4), are released. These gases are then excreted from the body into the atmosphere. Agriculture accounts for as much as 31% of the nation's CH_4 emissions (USDA, 2008) and is not only a source of greenhouse gases in the atmosphere, but also is potentially wasted metabolizable energy (Zhou, 2011). If this pathway became somehow blocked, the bacteria would have to allocate their waste products into other pathways. Ruminal bacteria release H_2 and CO_2 in the fermentation process (Figure 1), and methanogens use these to produce methane, thus maintaining equilibrium and permitting fermentation to continue (Figure 2). This regulates fermentation in the rumen by lowering the amount of hydrogen gas, allowing bacteria that produce hydrogen to continue metabolism. As fermentation continues in the rumen, the net production of NADH must be zero so methane or propionate can be produced to regenerate NAD. If the pathway favors propionate production, the amount of CH4 will decrease. As this happens, microbes shift the usable energy of NADH to latch H2 onto volatile fatty acids. 3-nitrooxipropanol (NOP) has been demonstrated to inhibit methanogenesis through inhibition of methane co-enzyme M reductase. (Reynolds, 2014) The inhibition of methanogenesis causes microbes to shift the reducing equivalent (H_2 and NADH) into production of other electron sinks or more reduced end-products. If the

methanogenesis pathway is nonfunctional, methane inhibition in bacteria permits the movement of aqueous hydrogen that helps in the production of longer chain fatty acids (Hristov, 2015). Longer chain fatty acids are used in bacteria to make the cell membrane more rigid, while branched chain fatty acids are used to make the cell membrane more flexible. A more flexible cell membrane allows a microbe to survive variable environments in the rumen, thus growing and multiplying. Brached chained volatile fatty acids also play a role in growth of microbes. BCVFA are required for growth in several strains of cellulolytic and non-cellulolytic rumen bacteria and are important to maintain membrane fluidity. (Wu and Palmquist, 1991). Supplementing BCVFA has been associated with increased fiber digestibility potentially by increasing microbial efficiency in the rumen (Wallace & Cotta, 1988). Since BCVFA are incorporated mainly into higher branched-chain fatty acids, aldehydes or their respective branched-chain amino acids (Allison, 1961) and these reactions release/use reducing equivalents, the effects of their supplementation could interact with methane inhibition, potentially increasing the formation of longer chain fatty acids like valerate and caproate.

With these findings in mind, I hypothesized there will be an increase in longer-chain fatty acid concentration when BCVFA is supplemented with NOP and fiber degradation will increase with BCVFA. Therefore, the overall objection of this project was to study the effect of BCVFA supplementation and its interaction with methane inhibition by NOP on the fermentation profile in continuous culture.

Materials and Methods

This study was conducted using cows from the Waterman Dairy Farm in Columbus, Ohio. Eight liters of rumen fluid was collected from four cannulated lactating dairy cows to ensure a good mixture of ruminant microbes. Rumen content were filtered through 2 layers of cheesecloth, then added to a mixture of buffer with clarified rumen fluid maintained at 39℃C until added to fermenters. Eight continuous culture fermenters were used in one period with 4 treatments. The treatment sums of squares were partitioned into a 2 x 2 factorial arrangement of treatments. The 4 treatments used in this project were a control fermenter with normal buffer added, a fermenter with BCVFA added, a fermenter with NOP added and a fermenter with both the inhibitor and BCVFA. Only 4 fermenters were equipped with gas measurement, but the effects of our NOP on methanogenesis are well known (Hristov, 2015). The trial continued for 11 days. The first 7 days of the trial were an adjustment period. During this period the fermenter were fed 100 g/d in two feedings with a 50:50 alfalfa:corn pellet ratio. (Table 1)

At the same time as the feeding, the treatments were given, 0.1mg of NOP and/or 7.2mmol/d of BCVFA. Every hour the pH of the fermenters, as well as the buffer drip, heat, and agitation were checked. Every two hours we checked the pH, the outflow of the fermenters, the pH of the buffer and the heat and agitation. After the adjustment period, there were 3 days of collection and 4 days of gas collection. We collected during the morning and in the evening before feeding and treatments. During these times we collected the solid outflow and the liquid outflow weighted them separately and combined them for sampling. The two outflows were combined, dried, and analyzed using the NDF procedure. Samples for VFA analysis where taken from the effluent and

were analyzed by Gas Chromatography with a packed column for VFA. Data were analyzed using PROC MIXED, with fermenter as a random effect.

Results

Looking at Table 2, we see that NOP increased longer chain fatty acids whereas BCVFA did not have an effect. However, there was an interaction such that when NOP and BCVFA were both added, in which the increase in longer chain wasn't as large as when only NOP was added. BCVFA and NOP together did not increase the BCVFA concertation, but separated they did; thus, the combination had a negative interaction and suggests that BCVFA is possibly being directed into longer chain FA. As expected, BCVFA had an increase in BCVFA concentration, which shows that our treatment was delivered to the appropriate fermenters. However, the BCVFA were not increased in the effluent, which can indicate that it was utilized by the microbes in the fermenter. The production of methane seemed to decrease with the addition of NOP (Figure 3). There are no results of the treatment of NOP and BCVFA because the methane reader going to this fermenter was not functional. In future trials, we would need to make sure the gas measuring lines are working. The effects of the treatments on NDF can be seen in Figure 4. There was a trend for BCVFA to increase NDF degradation. Many of the microbes, such as cellulolytic bacteria, require BCVFA as a growth factor because they can't make it themselves. These bacteria survive by getting some of the BCVFA from other microbes that can make it, which allows them to survive. While they can get some BCVFA from other microbes its low enough that it probably is inhibiting their growth and potential to degrade fiber, especially depending on the diet provided. If enough BCVFA

is supplemented it will allow them to grow in the rumen and degrade more fiber. NOP did not have an effect on the NDF degradation and there was no interaction between NOP and BCVFA on NDF degradation, more studies need to be done to prove that this is true. As for the longer chain fatty acids, there wasn't an effect of the treatments on the effluent of longer chain fatty acids (Figure 5). However, NOP has a trend to increase longer chain fatty acid outflow, which means the addition of these methane inhibiters can cause a change in how the microbes get rid of their H_2 and CO_2 in the form of tacking them on to the ends of fatty acids chains to make them longer. What is interesting is that the BCVFA treatment by itself did not increase the amount of longer chain fatty acids in the effluent. This may be due the microbes' protein needs. When the microbes are growing they need protein, so they may convert the BCVFA into branch chain amino acids (BCAA). Converting BCVFA to BCAA also uses reducing equivalences and competes with BCVFA to create longer chained fatty acids.

Conclusion

This project has shown that NOP does decrease the amount of methane in the fermenter. It is unknown if the interaction between NOP and BCVFA would lead to advances in environmental protection and potentially increase efficiency in the use of metabolizable energy. The addition of BCVFA may help stimulate fiber digestibility, which will increase feed efficiency in the cow but also likely increases bacterial growth and need for BCAA and longer chain FA. Thus, the effect on the live animal will have to be monitored. The interaction of BCVFA and NOP did not increase the amount of longer chain fatty acids present. This, however, was only one trial with 2 replications. Future

replications must be performed to create more accurate results. Future studies should assess NOP and BCVFA supplementation on live animals and its effects on overall animal efficiency and reducing feed cost. Overall, the benefits from a project like this may change the environmental impact of the industry as a whole while making it more profitable for the farmers.

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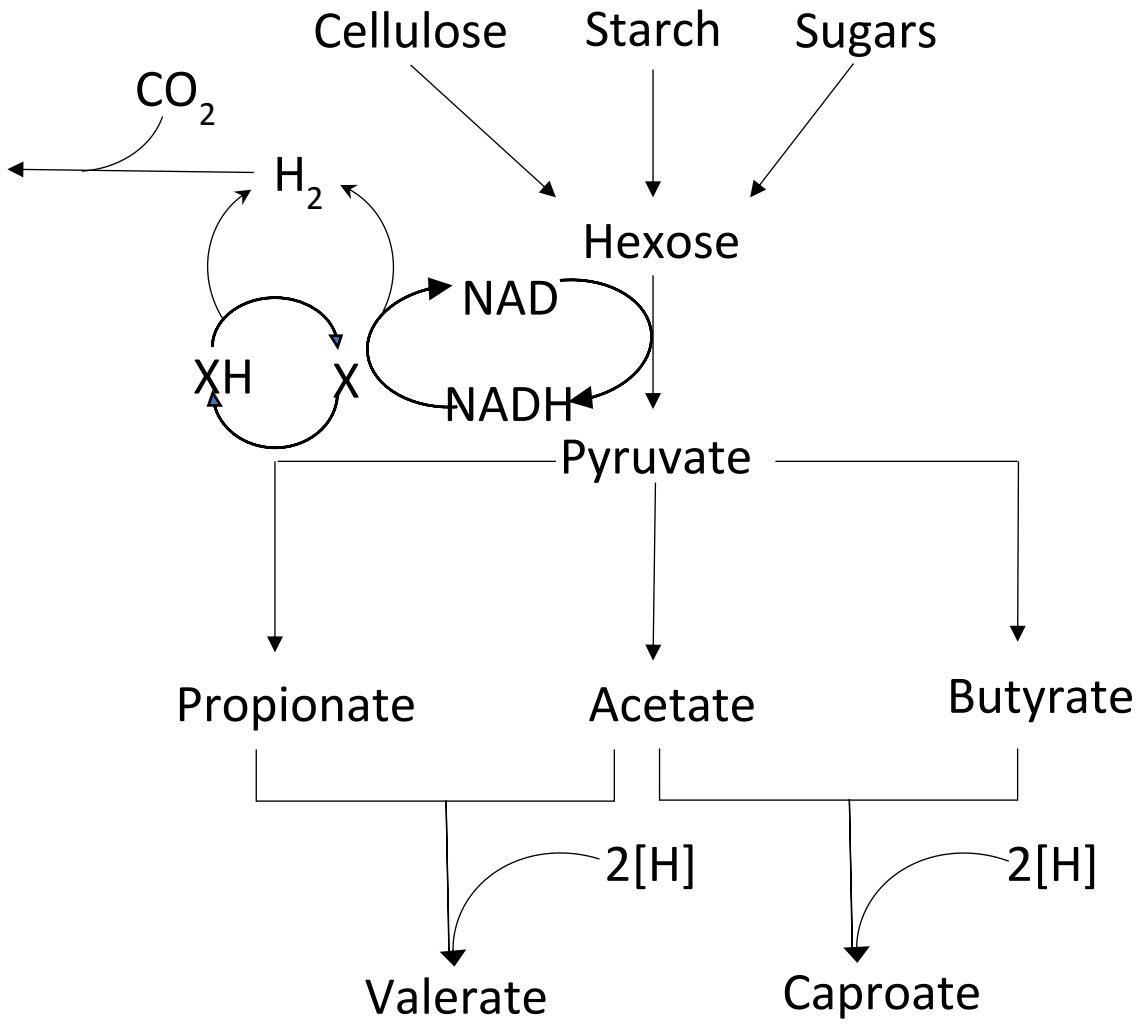

Figure 1: Metabolism of carbohydrates by ruminal bacteria (Russell, 2002)

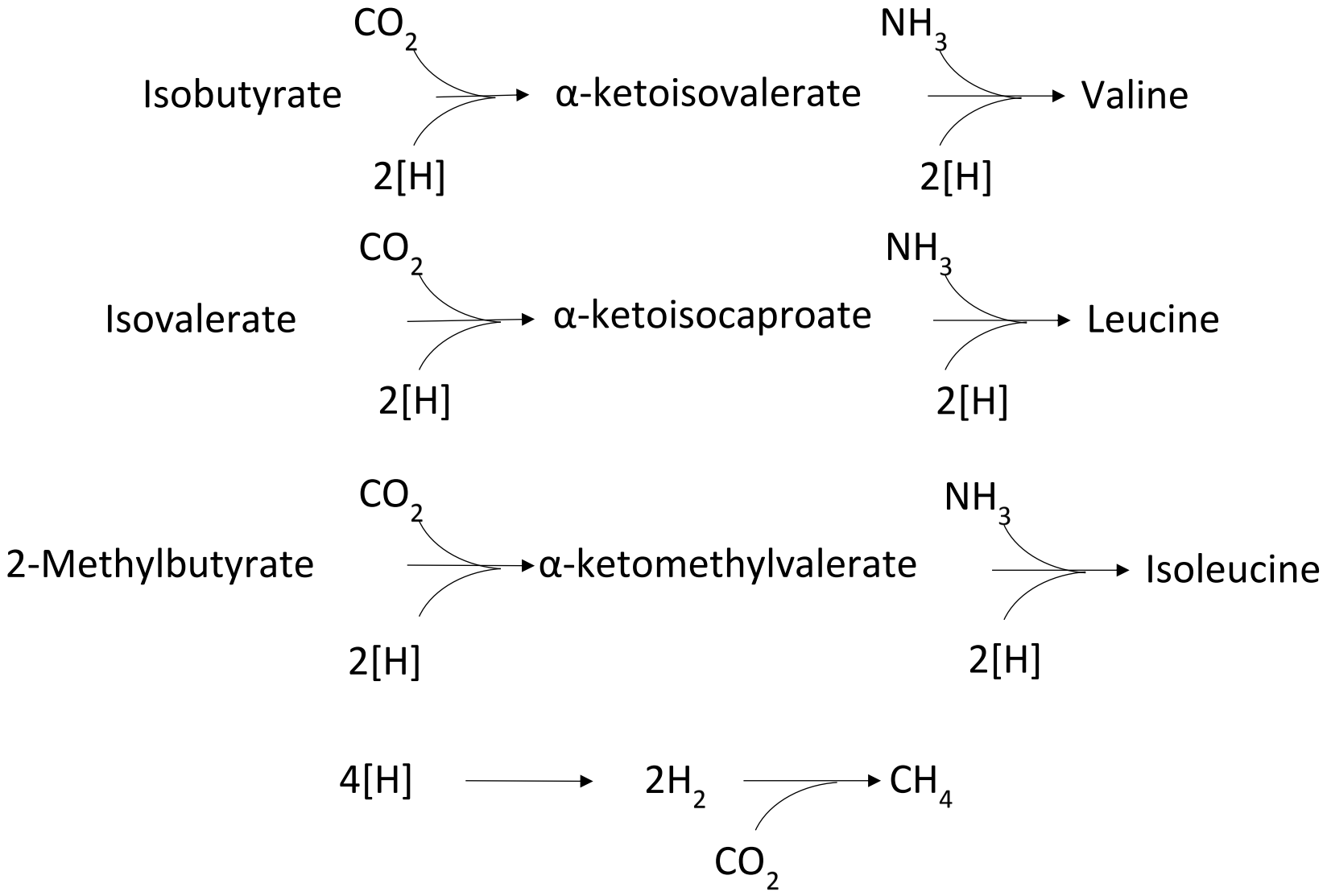

Figure 2: Deamination and decarboxylation of BCAA, and methanogenesis (Russell, 2002)

Item	
Diet ingredients, %	
Alfalfa Meal	50.0
Corn grain	32.3
Corn distillers grains w/ solubles	8.00
Soybean hulls	7.78
TM supplement	0.800
Soybean meal, 48%	0.500
Vegetable oil	0.420
Selenium 200	0.100
Magnesium oxide	0.100
Diet composition, %[1]	
CP	15.3
RDP (%CP)	51.2
NDF	33.6
Starch	24.4

Table 1: Nutritional Composition of Feed Pellets

	- CH4 Inhib.		+ CH4 Inhib.			P-Value		
	- BCVFA	+ BCVFA	- BCVFA	+ BCVFA	SE	CH4 Inhib.	BCVFA	Interaction
Longer Chain VFA Concentration, mol/100 mol Total VFA	1.68	2.16	2.37	2.08	0.11	<0.01	0.32	<0.01
BCVFA Concentration, mol/100 mol Total VFA	10.2	18.6	11.5	15.7	1.87	0.62	<0.01	0.18
BCVFA Effluent mol/d	28.9	41.1	41.6	33.7	5.41	0.66	0.73	0.16

Table 2: Results of each treatment and the interactions. (-) is the absences of a treatment and (+) is the presents of the treatment. Least Square means per treatment

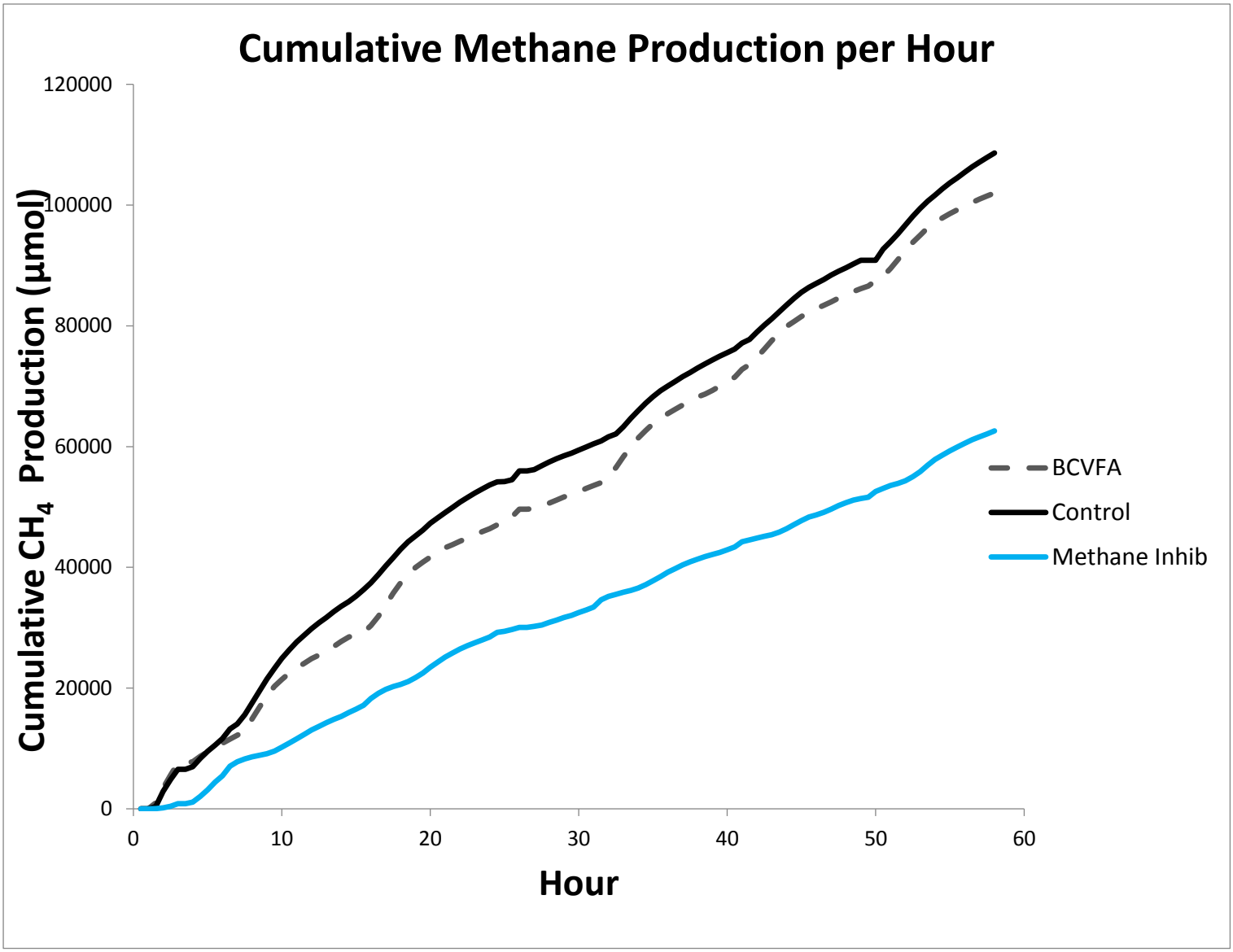

Figure 3: Cumulative CH_4 production per hour.

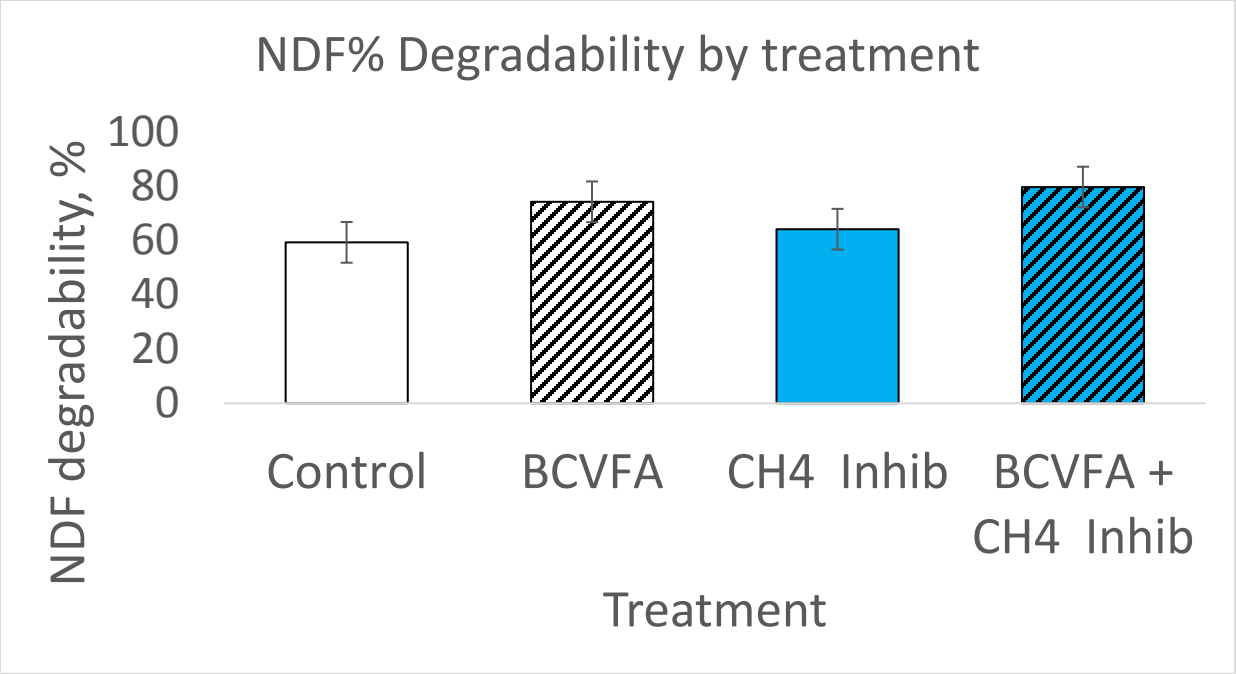

Figure 4: The main effect of BCVFA, CH_4 Inhib, and interaction *P* = 0.13, 0.54, and 0.97, respectively

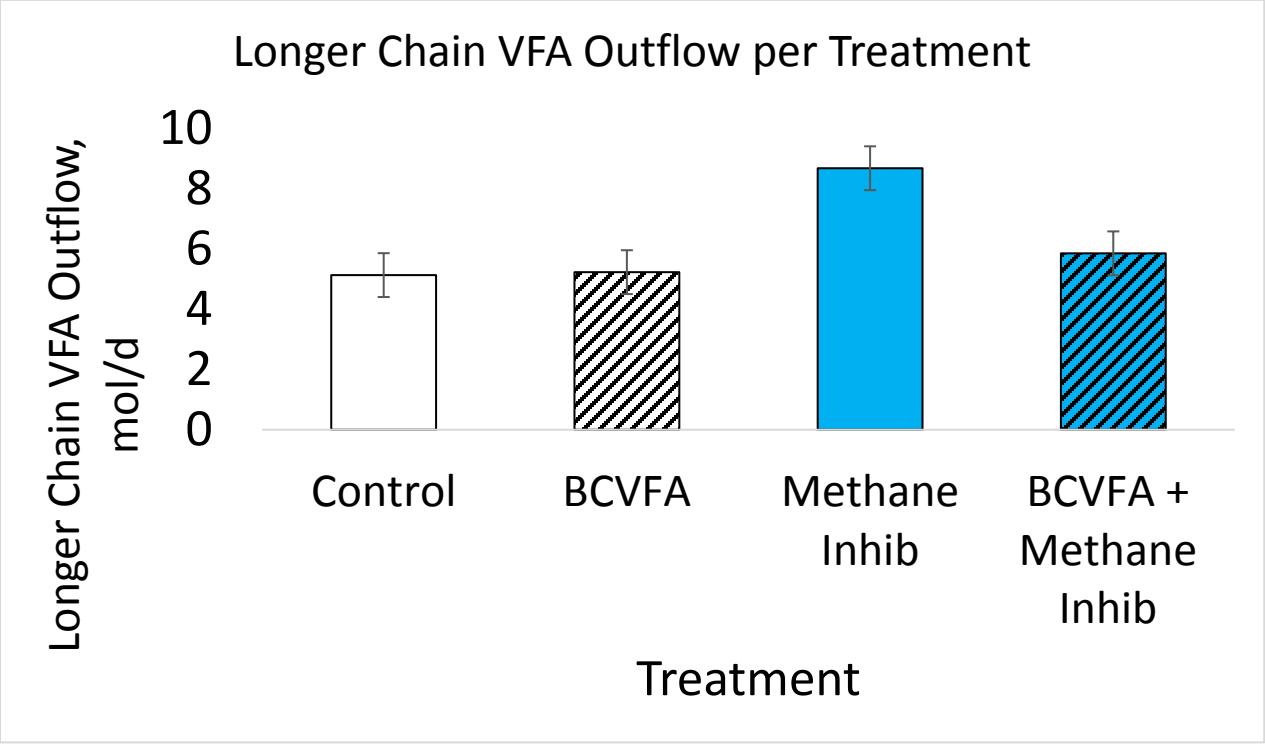

Figure 5: The main effect of CH_4 Inhib , BCVFA, and interaction *P* = 0.16, 0.06, and 0.14, respectively

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